PRODUCT INFORMATION & MANUAL

Potassium (K) Assay Kit (Colorimetric)

NBP3-25780

For research use only.
Not for diagnostic or therapeutic procedures.

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Potassium (K) Assay Kit (Colorimetric)

Catalog No: NBP3-25780

Method: Turbidimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.002 mmol/L

Detection range: 0.01-0.80 mmol/L

Average intra-assay CV (%): 1.1

Average inter-assay CV (%): 6.1

Average recovery rate (%): 94

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure Potassium (K) content in serum, plasma, milk, tissue, cells and other samples.

▲Background

Potassium ions are vital for the functioning of all living cells. The transfer of potassium ions across nerve cell membranes is necessary for normal nerve transmission; potassium deficiency and excess can each result in numerous signs and symptoms, including an abnormal heart rhythm and various electrocardiographic abnormalities. Fresh fruits and vegetables are good dietary sources of potassium. The body responds to the influx of dietary potassium, which raises serum potassium levels, with a shift of potassium from outside to inside cells and an increase in potassium excretion by the kidneys.

▲Detection principle

Under the alkaline condition, the sodium tetraphenylborate reacts with the potassium ions in the sample to form the potassium tetraphenylborate which is white and small particles with small solubility. Potassium tetraphenylborate particles are in a stable suspension state in the solution. The turbidity is proportional to the potassium ion concentration in the sample and potassium content can be calculated indirectly by measuring the OD value at 450 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Precipitant A	20 mL × 1 vial	2-8℃ , 12 months
Reagent 2	Precipitant B	1.25 mL × 2 vials	2-8℃ , 12 months
Reagent 3	Chromogenic Agent A	12.5 mL × 2 vials	2-8℃ , 12 months
Reagent 4	Chromogenic Agent B	Powder × 2 vials	2-8℃ , 12 months, shading light
Reagent 5	1 mmol/L Potassium Standard	1.25 mL × 2 vials	2-8℃ , 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Microplate reader(450 nm), Centrifuge, Micropipettor, Vortex mixer

Reagents

Deionized water

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

1. Hemolysis samples should not be adopted since red blood cells contain high concentrations of potassium ions.
2. Ammonia, mercury, chlorine can interfere with the determination of potassium ion.
3. It is recommended to use deionized water to prepare tissue homogenate and avoid potassium ion pollution.

Pre-assay preparation

▲Reagent preparation

1. Preparation of protein precipitant:
   Mix the reagent 1 and reagent 2 with the ratio of 8:1. Prepared the fresh solution before use.
2. Preparation of chromogenic agent:
   Dissolve a vial of reagent 4 with 12.5 mL reagent 3 and mix fully. Prepared the fresh solution before use.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

Sample requirements

1. Since there is a high concentration of potassium ions in red blood cells, the sample should avoid hemolysis.
2. Deionized water is the best homogenized medium for tissue homogenization to prevent the contamination of potassium ions.
3. Ammonium ion, heavy metal ion, chloride ion will affect the reaction, so the sample can't be added.
4. The samples are stable at 2~8℃ for 3~4 days and stable below -20℃ for several months.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.01-0.80 mmol/L).

The recommended dilution factor for different samples is as follows (for reference only)

Sample type	Dilution factor
Human serum	1
Rat serum	1
RAW264.7 cellular supernatant	1
Human plasma	1
Human milk	1
10% Rat liver tissue homogenization	2-4

Note: The diluent is deionized water .

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6'	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H: standard wells; S1-S80: sample wells.

▲Detailed operating steps

The preparation of standard curve

Dilute 1 mmol/L Potassium Standard with deionized water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8 mmol/L. Reference is as follows:

Number	Standard concentrations (mmol/L)	1 mmol/LStandard(μL)	Deionized water (μL)
A	0	0	250
B	0.1	25	225
C	0.2	50	200
D	0.3	75	175
E	0.4	100	150
F	0.5	125	125
G	0.6	150	100
H	0.8	200	50

The measurement of samples

(1) Preparation of supernatant: Mix the sample and protein precipitant with the ratio of 1:9 (For example, take 20 μL of sample and 180 μL of protein precipitant to mix fully). Centrifugate at 1100 g for 10 min. Take supernatant for detection.

(2) Standard well: Take 50 μL of standard solution with different concentrations to the wells.
Sample well: Take 50 μL of supernatant to the wells.

(3) Add 200 μL of chromogenic agent into the wells of Step 2.

(4) Cover the plate sealer, mix fully and stand for 5 min at room temperature.

(5) Measure the OD value at 450 nm with microplate reader.

▲Summary operation table

	Standard well	Sample well
Potassium standard solution with different concentrations (μL)	50	
Sample supernatant (μL)		50
Chromogenic agent (μL)	200	200
Mix fully and stand for 5 min. Measure the OD value at 450 nm.		

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. If the sample have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. The actual concentration is the calculated concentration multiplied by dilution factor.

The standard curve is: y= ax + b.

1. Serum (plasma) and other liquid sample:

$$\text{Potassium content (mmol/L)} = (\Delta A_{450} - b) \div a \times 10 \times f$$

2. Tissue sample:

$$\text{Potassium content (mmol/ gprot)} = (\Delta A_{450} - b) \div a \times 10 \times f \div C_{pr}$$

Note:

y: $OD_{Standard} - OD_{Blank}$. ($OD_{Blank}$ is the OD value when the standard concentration is 0)

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

10: Dilution multiple of sample in preparation of supernatant

f: Dilution factor of sample before test

C_{pr}: Concentration of protein in sample (gprot/L)

ΔA: Absolute OD ($OD_{Sample} - OD_{Blank}$)

Appendix I Data

▲Example analysis

Take 0.1 g of fresh rat liver sample, add 0.9 mL of 2-8℃ deionized water, then homogenize treat the sample in ice water bath, centrifuge at 10000 g for 10 min at 4℃ , then dilute the supernatant with deionized water for 2 times and carry the assay according to the operation table.

The results are as follows:

Standard curve: y = 0.77073x-0.00139, the average OD value of the sample is 0.404, the average OD value of the blank is 0.045, the concentration of protein in sample is 9.23 gprot/L, and the calculation result is:

K^+ content (mmol/gprot)= (0.404-0.045+0.00139) ÷0.77073×10×2÷9.23
=1.01 mmol/gprot

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

▲Plasma

Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80℃ for a month.

▲Milk sample

Collect the milk sample, centrifuge at 10000 g for 10 min at 4℃ and collect middle layer liquid for measurement.

▲Tissue sample

Take 0.02-1g fresh tissue to wash with homogenization medium at 2-8℃. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 1500 g at 4℃. Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

▲Cells

Collect the cells and wash the cells with homogenization medium for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (10^6): homogenization medium (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant. If not detected on the same day, the cells sample (without homogenization) can be stored at -80℃ for a month.

Note:

1. Homogenized medium: Deionized water.

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.
Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

(3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/ time, interval for 3 s, the total time is 5 min).

Appendix III References

1. Epstein W. The roles and regulation of potassium in bacteria[J]. Progress in Nucleic Acid Research & Molecular Biology, 2003, 75(1): 293-320.
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3. Adrogué H J, Madias N E. Sodium and potassium in the pathogenesis of hypertension[J]. N Engl J Med, 2007, 356(19): 1966-1978.
4. Christian D, Houda S, Christian R, et al. Protective effects of high dietary potassium: nutritional and metabolic aspects[J]. Journal of Nutrition, 2004, 134(11): 2903-2906.